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Gone Fishin' in 2005—Insights into the Inner Workings of Dendritic Cells

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Dendritic cells were discovered by Ralph Steinman and Zanvil Cohn a little more than three decades ago (Steinman and Cohn, 1973). I became interested in the topic about 20 y ago while I was a postdoctoral fellow. At that time, it was possible to read every paper that focused on dendritic cells, to "understand" the information that was presented, and to fit that information into a relatively simple conceptual framework. The subsequent recognition that dendritic cells are of critical importance in immunophysiology resulted in dramatically heightened interest in this fascinating cell type, and this in turn has resulted in an explosion of information that is increasingly difficult to become aware of and to synthesize, even for serious students of dendritic cells. This difficulty reflects not only the volume of the information that is published, but also its complexity. Since dendritic cells both initiate and shape T cell-dependent immune responses, studies of dendritic cell function are also studies of T cell biology and T cell function, with all the complexity that this entails. The article in the current issue by Mizumoto *et al* (2005) informs us that dendritic cell complexities extend to intracellular signaling pathways that are differentially engaged as dendritic cells respond to various stimuli. This complexity must also be carefully explored if we are to fully understand dendritic cells, and how to manipulate them for patient benefit. Because dendritic cell biology is a complicated topic, it may be helpful to put the studies of Mizumoto *et al* (2005) into context.

Dendritic cells are bone marrow-derived cells that can be found in all lymphoid, and almost all non-lymphoid, tissues. Dendritic cells in different tissues may differ from each other with regard to function as well as phenotype. A major function of dendritic cells is to initiate antigen-specific immune responses in naïve T lymphocytes and to influence the character of the immune responses that subsequently develop. Dendritic cells are also responsive to microenvironmental influences, and their functional properties are dependent on what is going on around them. Thus, the ideal way to explore dendritic cell function is to study dendritic cells *in situ* while they are participating in immune or inflammatory responses. Obviously, immune responses that are evolving *in vivo* are inherently complicated. Typically, *in vivo* experiments require painstaking development and characterization of animal models that often allow only highly focused questions to be addressed. In addition, definitive answers may be difficult to obtain. *In vivo* experiments involving human dendritic cells are problematic for other reasons. Thus, model systems that allow dendritic cell biology to be studied *in vitro* have been, and are, essential to make progress.

The field made a leap forward when a number of investigators developed methodology that allowed routine propagation of dendritic cells from human and murine blood- and bone marrow-derived precursors in primary cultures (Caux *et al*, 1992; Inaba *et al*, 1992a, b; Sallusto and Lanzavecchia, 1994). The availability of these cells for study in many laboratories around the world enabled rapid progress that underpins much of what we know about dendritic cells today. For example, homogeneous populations of dendritic cells derived from *in vitro* cultures allowed identification of physiologic dendritic cell agonists (including microbial products and cytokines), definition of cell surface receptors through which these agonists act, and detailed characterization of the ways dendritic cells influence T cell function.

Studies of dendritic cells that have been grown in primary cultures in laboratories have limitations, however. One limitation is that dendritic cells that are grown in different laboratories, or even in the same laboratory at different times, may have different characteristics. This frustrating occurrence probably reflects the sensitivity of dendritic cells to even minor changes in microenvironmental or culture conditions. In addition, the phenotypes of laboratory dendritic cells are not identical to even the tissue dendritic cells that they most closely resemble. Finally, dendritic cells grown in primary cultures are not amenable to certain kinds of experimental manipulations. Most notable are experiments that require introduction of genes of interest, such genes encoding active or inhibitory variants of signaling molecules, into dendritic cells by transfection or transduction.

Dendritic cell lines, including the XS106 cell line used in the paper by Mizumoto *et al* (2005), have their own utilities and limitations. Virtually unlimited capacity for growth and consistency over time are major benefits of cell lines. Despite the fact that the XS106 line was initially described more than 5 y ago (Timares *et al*, 1998), its major characteristics have not changed. Some dendritic cell lines, including the XS106 cell line, remain responsive to known dendritic cell agonists, making them suitable for cell activation studies such as those described in the Mizumoto paper. Most dendritic cell lines retain some dendritic cell surface markers as well as the ability to initiate antigen-dependent activation of T cells, and this is certainly true of XS106 cells. With regard to limitations, the degree to which dendritic cell lines can be activated is typically attenuated as compared with that of dendritic cells in tissues or dendritic cells in primary cultures. In addition, the extent to which dendritic cell lines resemble tissue dendritic cells is even more limited than it is for dendritic cells that are generated in primary cultures. Finally, it is generally accepted that dendritic cells in tissues are "end stage" cells that have lost their proliferative potential. Although the XS106 cell line retains

growth factor (GM-CSF and M-CSF)-dependence, its proliferative capacity far exceeds that of tissue dendritic cells. This latter characteristic of XS106 cells may be particularly relevant for, and potentially a confounder of, studies of transcription factor activity such as those described in this issue.

Mizumoto and coworkers are well aware of both the positive and negative attributes of the cells used in their studies. Over the past ten years, Akira Takashima's group has repeatedly and creatively used dendritic cell lines that they have developed and characterized dendritic cell lines to initiate studies of fundamental aspects of dendritic cell biology that they have subsequently confirmed in normal cells or in tissues (Xu *et al*, 1995). These investigators have also used dendritic cell lines to perform proof-of-concept experiments of novel dendritic cell-based therapies that have efficacy in animal models (Timares *et al*, 2003; Matsue *et al*, 1999). This issue of the *JID* reports yet another innovative and careful study from this group that could only have been carried out with a dendritic cell line.

Although microbial products, cytokines and growth factors, exogenous chemicals, and endogenous cellular metabolites that activate dendritic cells have been catalogued and much is known about receptors that are engaged by these dendritic cell agonists, information about how intracellular responses to these agents are integrated is limited. Typically, elucidation of the importance of signal transduction pathways in biological responses has involved making specific predictions, choosing appropriate pharmacologic activators or inhibitors (or mutated genes that encode active or inhibitory members of the pathway(s) of interest), introducing them into cells, and determining whether or not the intervention chosen has the predicted effect.

The study by Mizumoto *et al* (2005) is distinctly different in that the experimental approach does not require prediction of outcomes. Rather, the authors have systematically cataloged the involvement of 15 different transcription factors in response to 14 different known dendritic cell agonists in a totally unbiased way. Not so long ago, a study like this might have been criticized for being a "fishing expedition" that was not "hypothesis driven". Interestingly, in 2005, we have come to regard this kind of investigation as "hypothesis generating" and, based on the power of the approach and the frequency with which it is successful, to value it. The agonists chosen for study include microbial products, endogenous metabolites, and exogenous agents that are known to activate dendritic cells *in vivo* as well as *in vitro*. Transcription factor activity was assessed by introducing reporter gene expression constructs that are comprised of cDNAs that encode firefly luciferase, a protein that can be readily quantified via a sensitive chemiluminescence assay, located downstream of well characterized enhancer elements into the XS106 dendritic cell line using standard transfection methodology. Previous studies involving other cells had determined that increased production of luciferase in cells transfected with the individual constructs was a good surrogate marker of activation of the selected transcription factor.

The paper by the Takashima group is clearly written, so there is no need to detail the results. To summarize briefly, the paper describes overlapping and non-random utilization of arrays of transcription factors in response to the various agonists tested. Using hierarchical clustering analysis, agonists using the same or similar transcription factors could be grouped. The largest cluster is broad, meaning that there is incomplete overlap in the transcription factors that are utilized, and includes responses to most dendritic cell stimulators. More studies will be required to determine whether or not this cluster can be additionally subdivided. In addition to and in light of the limitations of *in vitro* studies and studies of dendritic cell lines that were discussed above, it will be important to carry out selected *in vivo* experiments to support or refute major conclusions of this study. Regardless of the outcome of these *in vivo* studies, however, the experimental system described by Mizumoto *et al* (2005) will be very useful. For example, it may constitute a platform that will facilitate directed or non-directed high throughput testing of agents that could become dendritic cell modulating drugs that will ultimately be useful in patients with cancer, chronic infections, or autoimmune diseases. In addition, the experimental system may prove to be useful as a component of a screen that could be used to assess the possible irritancy or potential antigenicity of environmental contactants, topical medications, or cosmetics.

For those who are actively engaged in laboratory investigations, the paper also serves as a reminder that there is great value in devoting time to actively think about innovative ways to utilize existing reagents and methodologies to ask important new questions. I know that Dr Takashima's group includes a number of avid fishermen, including Dr Takashima himself. I cannot help but wonder if their appreciation for some of the finer things in life, like cold beer and time spent in a boat on a lake or in the Gulf with live bait or a lure in the water, has something to do with their sustained creativity. Perhaps it is these interludes that enable them to do their best work, and the sign on the door that says "Gone Fishin'" is a prelude to the next series of interesting investigations.

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